ABSTRACT OF THE DISCLOSURE

In vitro methods for making a recombinant adenoviral genome, as well as kits for practicing the same and the recombinant adenovirus vectors produced thereby, are provided. In the subject methods, the subject genomes are prepared from first and second vectors. The first vector includes an adenoviral genome having an E region deletion and three different, non-adenoviral restriction endonuclease sites located in the E region. The second vector is a shuttle vector and includes an insertion nucleic acid flanked by two of the three different non-adenoviral vectors present in the first vector. Cleavage products are prepared from the first and second vectors using the appropriate restriction endonucleases. The resultant cleavage products are then ligated to produce the subject recombinant adenovirus genome. The subject adenoviral genomes find use in a variety of application, including as vectors for use in a variety of applications, including gene therapy.